[0357] Then, the working electrode substrate was washed with TBS-T. Then, 2 mg/mL of streptoavidin [manufactured by Vector Laboratories] (the second conjugate) was added to TBS-T so that its concentration was 4 μg/mL. 30 μL of the obtained mixture was poured into the above space. Thereafter, the working electrode substrate was incubated at 25° C. for 30 minutes. Thus, streptoavidin was bound to the biotin-labeled anti-human interleukin-6 antibody or the anti-human interferon-γ antibody on the working electrode.

(1-3) Dye-Labeling

[0358] The working electrode substrate subjected to the process (1-1) was washed with TBS-T. Then, TBS-T was added to 100 μL of a solution containing the biotinylated-DNA/Alexa Fluor 750-labeled DNA complex obtained in Preparation example 2-5 (concentration of the complex: 93 μg/mL) in an amount 10 times the amount of the solution. Thereafter, 30 μL of the obtained mixture was poured into the above space. Thereafter, the working electrode substrate was incubated at 25° C. for 30 minutes. Thus, a complex containing the analyte (a detecting object), the first conjugate, the second conjugate, and the labeled form was formed on the working electrode. The complex formed of the first conjugate and the second conjugate corresponds to the binding substance in the label binding substance obtained in Example 2-1. That is, on the working electrode, Alexa Fluor 750 as the labeling substance is bound to the binding substance bound to the analyte via DNA as the modulator.

(2) Measurement of Photocurrent

[0359] Silicone rubber was placed around the working electrode substrate so that a 0.2-mm-thick side wall was formed. Then, the space surrounded by the working electrode substrate and the silicone rubber was filled with the electrolytic solution obtained in Preparation example 2-3. The space filled with the electrolytic solution was sealed with the counter electrode substrate obtained in Preparation example 2-4 from the upper side of the working electrode substrate. Thus, the working electrode and the counter electrode are brought into contact with the electrolytic solution. Then, the detection chip including the working electrode substrate and the counter electrode was placed in an electrochemical measurement device. The working electrode lead and the counter electrode lead were connected to the ammeter.

[0360] The light source (wavelength: 781 nm, laser light source with an output power of 13 mW) emits excitation light from the working electrode substrate side toward the counter electrode substrate. The labeling substance Alexa Fluor 750 is excited by photoirradiation, thereby generating electrons. When the generated electrons are transported to the working electrode, current flows between the working electrode and the counter electrode. Then, the electric current was measured. Current measurement was performed on an anti-interleukin-6 antibody-immobilized portion and an anti-interferon-γ-antibody-immobilized portion. The operation was performed in the same manner as described above except that the analyte was not used. The control experiment when the analyte was not present was performed.

[0361] FIG. **38** shows the examined results of a relationship between the kind of the detection subject and photocurrent in Example 2-6.

[0362] From the results shown in FIG. **38**, it is found that when the analyte is interleukin-6, the photocurrent detected in the anti-interleukin-6 antibody-immobilized portion is 0.20 nA and the photocurrent detected in the anti-interferon-γ-antibody-immobilized portion is 0.08 nA, while when the photocurrent is detected in the absence of the analyte, the photocurrent detected in the anti-interleukin-6 antibody-immobilized portion is 0.06 nA and the photocurrent detected in the anti-interferon-γ-antibody-immobilized portion is 0.07 nA. It is found that when the analyte is interferon-γ, the photocurrent detected in the anti-interleukin-6 antibody-immobilized portion is 0.09 nA and the photocurrent detected in the anti-interferon-γ-antibody-immobilized portion is 0.14 nA. These results suggest that it is possible to detect an analyte specific to the antibody which is the trapping substance immobilized on the same electrode.

[0363] On the other hand, it is found that when the analyte is a mixture of interleukin-6 and interferon-γ, the photocurrent detected in the anti-interleukin-6 antibody-immobilized portion is 0.17 nA and the photocurrent detected in the anti-interferon-γ-antibody-immobilized portion is 0.10 nA. These photocurrents are larger than those in the absence of the analyte, however, they are smaller than the photocurrent detected when the analyte is a single interleukin-6 or a single interferon-γ. Therefore, it is found that when the detecting object is a mixture of a plurality of types of analytes, it is possible to specifically detect each analyte, however, a slight decrease in detection sensitivity is observed as compared with the case where a single analyte is detected.

[0364] The above results suggest that it is possible to simultaneously detect various kinds of analytes on the same electrode by using a multivalent-labeled binding substance in which more labeling substances are immobilized to the binding substance through the interaction between modulators in order to label the analyte.

[Sequence Listing Free Text]

[0365] SEQ ID NO: 1 is a sequence of maleimidized DNA.

[0366] SEQ ID NO: 2 is a sequence of labeling substance-retaining DNA.

[0367] SEQ ID NO: 3 is a sequence of Alexa Fluor750-labeled DNA.

[0368] SEQ ID NO: 4 is a sequence of CK19 DNA-trapping DNA.

[0369] SEQ ID NO: 5 is a sequence of CK19 DNA.

[0370] SEQ ID NO: 6 is a sequence of CK19 recognizing/label-retaining DNA.

[0371] SEQ ID NO: 7 is a sequence of CK19-recognizing DNA.

[0372] SEQ ID NO: 8 is a sequence of label-retaining DNA-binding DNA.

[0373] SEQ ID NO: 9 is a sequence of Alexa Fluor750-labeled CK19-recognizing DNA.

[0374] SEQ ID NO: 10 is a sequence of DNA used as a modulator in Examples 2-1 and 2-2.

[0375] SEQ ID NO: 11 is a sequence of biotinylated-DNA. The 5' terminal of phosphate group is a biotinylated adenine base through the $(CH_2)_3$ linker

[0376] SEQ ID NO: 12 is a sequence of Alexa Fluor 750-labeled DNA.